REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	07/30/02	Final Report 09/15/2000-09/14/2001

4. TITLE AND SUBTITLE
Miniature Bioprocess Array: A Platform for Quantitative Physiology and BioProcess Optimization

5. FUNDING NUMBERS
N00014-00-1-0948

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Office of Naval Research
800 N. Quincy St.
Arlington, VA 22217-5000

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution Unlimited

20020805 072

13. ABSTRACT *(Maximum 200 words)*

We have developed a high-throughput miniature bioprocess array for the cultivation of microorganisms in a controlled, reproducible environment. The miniature bioprocess array is based on an array of 150-ul wells, each one of which incorporates MEMS for the closed-loop control of cell culture parameters such as temperature, ph, and dissolvedoxygen. The wells incorporate a suite of sensors, including interdigitated capacitors for cell density, thermopile temperature sensors, and oxuten sensors. Deep reactive-ion teched (DRIE) capacitive sensors enable new capabilities, such as the measurement of cell density in the bulk of the solution. Oxygen is generated by electrolysis, which also provides a means fo mixing the solution in the well. Data acquisition, communication, and control will be implemented in foundry CMOS. A four-wire bus connects the electronic interface at each well to each other and to a battery, a clock serial input/output, and ground.

14. SUBJECT TERMS		15. NUMBER OF PAGES
Cultivation,	Bioreactor, assay, 96-well plate	5
		16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

Final Report

GRANT #: N00014-00-1-0948

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INSTITUTION: University of California at Berkeley

GRANT TITLE: Miniature Bioprocess Array: A Platform for Quantitative Physiology and BioProcess Optimization

AWARD PERIOD: 09/15/2000 - 09/14/2001

OBJECTIVE: The goal of this work was to develop a high-throughput miniature bioprocess array and complementary models of gene expression to assess to effects of changes in culture parameters or drug targets on cell physiology. The objectives were (1) to develop an array of 150-µl wells, each one of which incorporates MEMS for the closed-loop control of cell culture parameters such as temperature, pH, and dissolved oxygen; (2) to test the effect of changes in culture conditions on production of metabolic by-products and gene expression in *Escherichia coli* by measuring cell density, substrate consumption, and product formation, as well as gene expression as a function of culture condition using gene chips; and (3) to integrate the data for gene expression, metabolite production, substrate consumption, and cell growth with existing models of gene expression and metabolism.

APPROACH: The miniature bioprocess array is based on an array of 150-µl wells, each one of which incorporates MEMS for the closed-loop control of cell culture parameters such as temperature, pH, and dissolved oxygen. The wells incorporate a suite of sensors, including interdigitated capacitors for cell density, thermopile temperature sensors, and oxygen sensors. Deep reactive-ion etched (DRIE) capacitive sensors enable new capabilities, such as the measurement of cell density in the bulk of the solution. Oxygen is generated by electrolysis, which also provides a means of mixing the solution in the well. Data acquisition, communication, and control will be implemented in foundry CMOS. A four-wire bus connects the electronic interface at each well to each other and to a battery, a clock, serial input/output, and ground.

Initial research has explored the precision possible with a plastic assembly substrate that can be laminated between two microtiter plates. This plastic layer is intended to provide dimensional stability that is superior to that of the injection molded plastic used for the microtiter plate. Key issues are strategies for multi-step or simultaneous multi-part assembly, planarization processes that are compatible with the plastic substrate, and the interconnect metallization process.

The miniature bioprocess array is being tested with *Escherichia coli*. As we hope to test the effect of various cultivation conditions on *Escherichia coli* global cellular gene expression, we are developing DNA arrays for *E. coli*. We have amplified all 4,290 genes in the *E. coli*

genome and are currently printing slides for analysis of *E. coli* gene expression.

ACCOMPLISHMENTS: We have developed an 8 x 1 array of bioreactor wells. The wells were constructed from processed silicon wafers and industry standard 96-well microtiter plates. Each well contains an independently addressable microfabricated sensor and actuator suite that provides closed-loop control over temperature, generates oxygen electrochemically, and provides cell density data. In addition, we have amplified all 4,290 genes in the *E. coli* genome and are starting to print DNA arrays for analysis of global cellular genes expression in the miniature bioprocess array.

Microfabrication starts with p-type silicon wafers. An 800-nm silicon nitride film is deposited. A 1.5-μm p-type polysilicon film is then deposited and patterned. A second 1-μm silicon nitride film is deposited and patterned with vias to the polysilicon. Finally, a 2000-Å Ti/Pt film is sputtered and patterned. Wafers are then diced into strips of eight chips. Each reactor chip has a 14 mm x 9 mm footprint; a single 8-chip strip has dimensions 14 mm x 72 mm.

To assemble an 8 x 1 reactor, an 8-chip strip is first bonded to a 1 mm thick glass slide using two-part commercial epoxy. Once the epoxy is dry, grooves are cut into the silicon chips. These grooves provide some degree of thermal isolation between reactors. The silicon strip is then affixed to a custom built fan-out board and wire-bonded. Next, commercial 96-well microtiter plates are separated into rows of eight wells; 2 mm holes are then drilled through what would have been the bottom of each well. A row of eight wells is bonded to the silicon strip using aquarium grade silicone adhesive. The adhesive dries in 15-24 hrs. Epoxy is *not* a suitable adhesive for sealing and bonding reactor wells; in aqueous solutions, excess amines present in epoxy hardeners give rise to a very high pH (>10) even in buffered medium.

Our custom-built fan-out board fits into a module containing conditioning, amplification, and interface electronics. Data acquisition is performed using a National Instruments DAQCard AI-16E-4 and a laptop computer. All codes were written in MATLAB.

Each chip contains one polysilicon serpentine thermistor (5KΩ) and three polysilicon heaters (400Ω). The Ti/Pt bond pads contact the polysilicon through vias outside the well footprint to avoid electrical contact with the aqueous environment in the well. Temperature control is done in ON/OFF fashion (i.e., there is no proportional power control to the heaters) with FET switches controlled by DAQ board digital lines (V=25 volts DC). Although this does give rise to oscillating temperatures, it is sufficient to provide ±1°C control. The eight thermistors are multiplexed into a Wheatstone bridge with a reference resistor (on-board); a DAQCard analog input reads the output of the bridge directly. Calibration was performed using two commercial thin-wire thermocouples (Omega), one inserted under the silicon strip and another immersed inside the well.

Thermal coupling between wells can be significant and depends in part on the style of microtiter plate used. Plates whose wells are molded from a single piece of plastic tend to have significant thermal

coupling. Certain microtiter plate designs (e.g., a single row of 8 wells from a 96-well plate) have wells connected by thin plastic strips. Using these, the main source of thermal coupling is the underlying silicon strip. To reduce this, grooves are diced into the chip as described above.

Each reactor contains three sets of Ti/Pt electrolysis electrodes. Each set consists of a 10 x 10 array of 25 μm x 25 μm Ti/Pt squares with 25 μm spacing. Rows of the array are biased such that they alternate between cathode and anode bias. In pure water, with a potential just above 2 V, one molecule of O_2 is generated at the anode for every four electrons flowing between cathode and anode. *E. coli* typically consumes oxygen at a rate of 40 mmol O_2/gram Dry Cell Weight/hour (Atkinson 1991). The current needed to supply this demand is 0.86 mA, and the rate of water consumption is 288 nL/hr, small enough to be of no concern for cultivation times <24 hrs.

The cell density sensor was calibrated by pipetting known concentrations of liquid cell culture into wells. Each reactor well was filled with 200 μl of liquid suspension from a shake flask culture. Initial and final densities were also confirmed with a spectrophotometer. Specific growth rate, μ, was calculated for the exponential phase of growth. The reactor assembly was agitated on a shaker table at 150 rpm. As expected, the optimal growth temperature is near 37°C.

CONCLUSIONS: In summary, the thermistor/heater(s) system and the cell density sensor were successfully implemented. In the thermal control loop, thermal coupling can set substantial limitations on the temperature spread achievable in an NxN system. A better design would use conical microtiter plates (such as centrifuge tubes); these are isolated from each other fairly well by the surrounding ambient. The capacitive cell density sensor should be re-designed in a 4-electrode configuration in order to eliminate polarization effects. Additionally, a circuit containing a lock-in amplifier is being designed to distinguish between the resistive and reactive components of the impedance.

Using electrolysis to provide oxygen to the cells proved to be a challenging problem. The voltages required to generate the oxygen flows of interest also give rise to reactive oxygen species (primarily ozone) that have strongly bactericidal effects. However, the new design eliminates the generation of ozone.

SIGNIFICANCE: This device should allow the cultivation of environmental microorganisms that have been difficult or impossible to cultivate using standard methods. In addition, this device should be useful for optimizing cell cultures as one will have a controlled environment in each well of a high throughput device.

PATENT INFORMATION: Two patent applications have been filed, one for the oxygen-generating electrolysis device and one for the assembled apparatus.

AWARD INFORMATION: N/A

PUBLICATIONS AND ABSTRACTS

1. M. M. Maharbiz, R. T. Howe, J. D. Keasling, "Silicon Microbial Bioreactor Arrays," Proceedings of the IEEE-EMBS Special Topic Conference on MicroTechnologies in Medicine & Biology," Paris, France, October 12-14, 2000. pp. 165-170.
2. M.M.Maharbiz, W.J.Holtz, S.Sharifzadeh, J.D.Keasling and R.T.Howe. 2002. "A microfabricated electrochemical oxygen generator for high-density cell culture arrays." Solid-State Sensor, Actuator and Microsystems Workshop, Hilton Head Island, SC, June 2-6 2002. pp.259-264.